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PARTICLE TRANSPORT AND INCORPORATION DURING SKELETON FORMATION IN A KERATOSE SPONGE: *DYSIDEA ETHERIA*

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Abstract

The mechanism and significance of sand particle incorporation into skeletal fibers were investigated in the sponge, *Dysidea etheria.* Time lapse cinemicrography of the sponge surface showed that this species actively transported particles at an average rate of 7.5 μm/min to areas where skeletal fibers were formed. Transport was associated with specific structures of the sponge's dermal membrane. Sand particles applied to sponge explants for five weeks increased skeletal fiber growth over growth in explants experiencing ambient particle loads. Explants deprived of particles had significantly less fiber growth. The location of applied particles influenced the direction of fiber growth. The results indicate that particles enhance skeletal fiber growth by acting as a filler material in the construction of fibers and may influence the overall direction of growth and shape of the skeleton.

Introduction

Sponges build a wide variety of skeletal structures. Many demosponges supplement their spongin skeletons with particulate material acquired from the environment, and the regular presence of particles in the skeletal fibers of some sponges has been used as a taxonomic character in sponge identification (*e.g.,* Lendenfeld, 1889, de Laubenfels, 1950). The incorporation of macroscopic pieces of foreign material in a supporting structure is an unusual feature that appears to be unique to sponges. Other organisms, like polychaete worms, use sand to build protective tubes, but such structures are outside the body of the animal whereas the sponge's skeletal fibers are internal. Thus, unlike their use in the worm tube, the incorporation of particles during skeletogenesis in the sponge must be closely coordinated with the sponge's growth and morphogenesis.

Previous discussions of particle incorporation in sponges addressed mechanisms of particle selection (Schulze, 1879, Lendenfeld, 1889, Sollas, 1908) and functions of particles in support and skeletogenesis (Shaw, 1927). This work relied on the study of fixed material which did not allow direct observation of the processes of particle in-incorporation and skeletal growth. Although these studies clearly showed that particles were associated with skeletal fibers, sometimes in very regular patterns, little was known of the pathway of the particle as it moved from the environment into the skeletal fibers. In addition, the observed associations of particles with fibers did not unequivocally demonstrate their requirement or role in skeletogenesis (see *e.g.,* Shaw, 1927). The purpose of the present study was to elucidate the process of particle incorporation and to investigate the role of particle incorporation in skeletal growth. Time lapse

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cinemicrography was used to directly observe the slow processes of particle sorting on the surface of the sponge that led to incorporation of particles into growing skeletal fibers. Field manipulations were performed to determine the effect of particle availability on sponge fiber growth.

Materials and Methods

Organism of study

The sponge studied was the keratose demosponge, *Dysidea etheria* de Laubenfels (Order Dictyoceratida). This species builds a skeletal network of spongin fibers that can be filled with a wide variety of foreign particles. Particles are restricted mainly to the radially arranged primary skeletal fibers. Secondary skeletal fibers crosslink the primary fibers to form a skeletal meshwork. The apices of the primary fibers abut on the dermal membrane that forms the surface of the sponge and create cone-shaped protrusions on the surface called conules (Fig. 1). The loose construction and relative transparency of this sponge allows the direct observation of interactions between the outer surface and underlying distal skeletal elements in the living material. The present study focused on growth of the primary skeletal fibers at the conules.

Collection and filming of sponges

All observations on living material were performed at the Bermuda Biological Station. Live sponges were collected with their basal substratum intact to minimize disturbance to the animal and were maintained in a Plexiglas flow through tank (flow rate = 1 cm/s) to obtain as normal a behavior as possible. Sponges were filmed at one frame per minute with a 16 mm Bolex movie camera using Kodak Plus-X 7276 reversal film. The camera was mounted over the phototube of a Wild M5 stereomicroscope (field of view 9 by 11 mm) and sponges were filmed overnight for seven to nine hours under constant illumination of a fiber optics lamp. Calcium carbonate sand particles (average diameter 112 μm, range 67–201 μm) were sprinkled on the surfaces of the sponges and several frames were shot to note the initial location of particles before time lapse filming began. Seven sequences were filmed but only one 7.5-hour sequence was analyzed in detail.

Frame-by-frame analysis was performed using a NAC Inc. DF-16C projector. Particle movement was analyzed in five circular conule areas and five randomly chosen rectangular nonconule areas (Fig. 1). Directions and rates of movements of individual particles on the sponge surface were measured from tracings of particle outlines every ten frames (= ten minutes). Vectorial representations of particle movement were produced by connecting the centers of sequential particle outlines (see Fig. 3). Directions of movement were measured by the angles formed between the line segments and could vary from −180 degrees to the left to +180 degrees to the right, with a zero angle indicating no change in direction of movement. To test if directions of particle movements were random, observed particle movements were compared to expected movements of randomly directed particles (having equal probability of moving in any direction) by comparing the distributions of observed angle values to the expected distribution generated by random movement using the Chi-square goodness of fit test (Sokal and Rohlf, 1969). Rates of particle movement were estimated from linear regressions of particle movement with time. Particle position was measured as the distance between the particle and the conule apex.

Skeletal growth experiments

The skeletal growth experiments were performed for five weeks in Walsingham Pond, Bermuda. Explants and whole sponges were used in experimental treatments. Explant samples were made by cutting whole sponges into 1 cm^3 pieces and tying the pieces onto glass coverslips with sewing thread. The coverslips were mounted on Plexiglas holders and attached to metal racks. There were seven to ten explant replicates per treatment. Explants were allowed to heal in the field for one week before beginning experimental treatments. Siliceous sand, which was distinguishable from the experimental sand, was applied to all sponges two days before the experiment and was incorporated into the skeleton to serve as a common reference point from which skeletal growth was measured.

There were five manipulations of particle availability in the experiment: three particle addition treatments and two particle depletion treatments. In the addition treatments, calcium carbonate sand particles of three different sizes, obtained by crushing and sifting beach sand through a graded series of sieves, were applied to cleaned sponge surfaces every other day. The average diameters of the three size classes of sand were 112 μm (small), 250 μm (medium), and 540 μm (large). The white color of the carbonate sand differentiated it from indigenous Walsingham Pond sediments. The particle depletion treatments consisted of cages made of 100% nylon fabric (mesh size 40 μm) that enclosed the explants and excluded particles. Explants caged on alternate days (half caged) had 32% average dry weight of ambient particles excluded and explants kept constantly caged (fully caged) had 71% average dry weight of ambient particles excluded. Particle exclusion was assessed by collecting sediment samples in small plastic containers for three 5-day periods inside and outside of cages. Controls for all treatments were explants exposed to ambient particle loads. The secondary effects of cages were examined with half and fully caged explants exposed to medium particles and compared to uncaged explants treated with medium particles.

The effect of the location of particle application on skeletal growth was observed in three whole sponges that were turned on their sides prior to application of medium particles. Three control sponges were similarly reoriented and grown under ambient particle conditions.

At the end of the experiment, samples were collected, fixed for 2 days in 10% formalin with 0.5% CPC (cetylpyridinium chloride) to aid matrix preservation (Williams and Jackson, 1956) and transferred to 70% ethanol for storage. Samples were stained with saturated basic fuchsin in 95% ethanol, embedded in paraffin, and sliced into approximately 5-mm thick slabs. The slabs were deparaffinized and embedded in Caroplastic (Carolina Biological Supply Co.) which polymerized to a colorless material of high refractive index and allowed observation of the sponge skeletal fibers. Lengthwise growth of primary fibers was measured using a Wild M5 stereomicroscope. Fibers from sponges not treated with carbonate particles were measured from the intercalation of the siliceous marker sand. Fibers from sponges treated with particles were measured more easily from the intercalation of the more numerous carbonate particles. This point approximately coincided with the position of the siliceous sand.

The mean fiber length for each explant constituted an independent observation in one-way analyses of variance (ANOVA) for the effects of the treatments on fiber growth. Separate analyses were made for particle additions, particle depletions and cage effects. Multiple *t*-tests were performed when the ANOVA treatment effect gave a significant result. The Bonferroni inequality that adjusts confidence levels for multiple comparisons was applied to multiple *t*-tests. All statistical procedures were performed in SAS (Statistical Analysis System, 1982).

Results

Dermal membrane morphology and behavior

Figure 1 shows the morphology of the filmed area of the sponge. The dermal membrane of *D. etheria* is characterized by a reticulating pattern formed by thicker regions of the dermal membrane mesohyl. Radially oriented reticulations converge upon the raised conule apices where the primary skeletal fibers meet the dermal membrane; connecting reticulations between conules lack such orientation. The areas between reticulation lines in the dermal membrane are perforated by groups of ostia. Excurrent oscula arise from nonreticulated, smooth areas of the membrane.

Time lapse cinemicrography showed numerous dermal membrane contractions and changes in the appearance of reticulations. The contractions gave the membrane an oscillating appearance and contributed to particle movement. Reticulations were not initially distinguishable but gradually became more distinct about sixty minutes after particle application. Evidence of membrane contractions and changes in reticulation appearance were also observed in nonfilmed sponges.

Particle movement

Initially, particles were scattered randomly over the entire membrane surface. Within thirty minutes after application, most of the particles on conule areas had moved down the slopes of the conule sides, presumably by gravity, and collected at the bases of the conules (Fig. 2a). As the dermal membrane reticulations became more visible, scattered groups of two to ten particles aggregated into larger clumps and became aligned on the reticulations (Fig. 2b). The particle clumps could be maintained or could disaggregate during subsequent movement.

Slow movement of particle clumps was observed on reticulation lines after particle aggregation and alignment. Most movement was observed on the radial reticulations in conule areas, where particles moved from the base to the apex of the conule (Figs. 2b–e, 3). The particles became harder to distinguish as they neared the apex area, probably as a result of their engulfment into deeper layers of the sponge (Fig. 2f). Limited particle movement was also observed on connecting reticulations in nonconule areas but was more difficult to discern since particles often stopped at branch points. The diffuse branching pattern also made the net direction of movement more difficult to determine. Approximately 20% of the particles applied to the sponge moved slowly. Nearly all slow movement ended 200 minutes after particle application.

The slow movements of particles were easily differentiated from more rapid and erratic movements of particles caused by contractions of the dermal membrane (Figs. 2d, 3). Slow movement of particles maintained fairly constant rates averaging 7.5 $\pm$ 2.1 μm/minute (n = 15) (Fig. 4) and particles moved nonrandomly towards conule apices, as shown by comparison of the direction of movement with a random movement pattern (Fig. 5). By contrast, erratic movement of particles could occur more quickly (maximum rate 27.9 $\pm$ 5.5 μm/min, n = 4), was sustained for shorter periods of time (Figs. 2d, 4) and was random (Fig. 5). Henceforth, the slow movement of particles will be referred to as transport.

Not all particles stayed on the sponge surface. Occasional rapid disappearances could be detected on film and attributed to inhalation of particles. General observations of particle-treated sponges indicated that particles could be consolidated in mucus clumps and sloughed off the sponge surface.

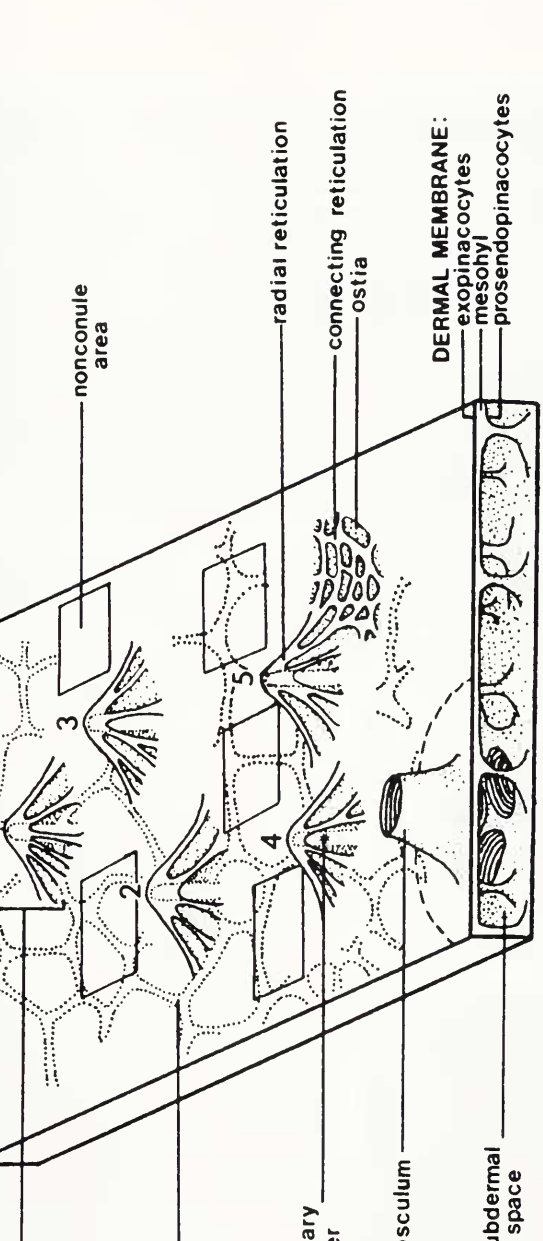
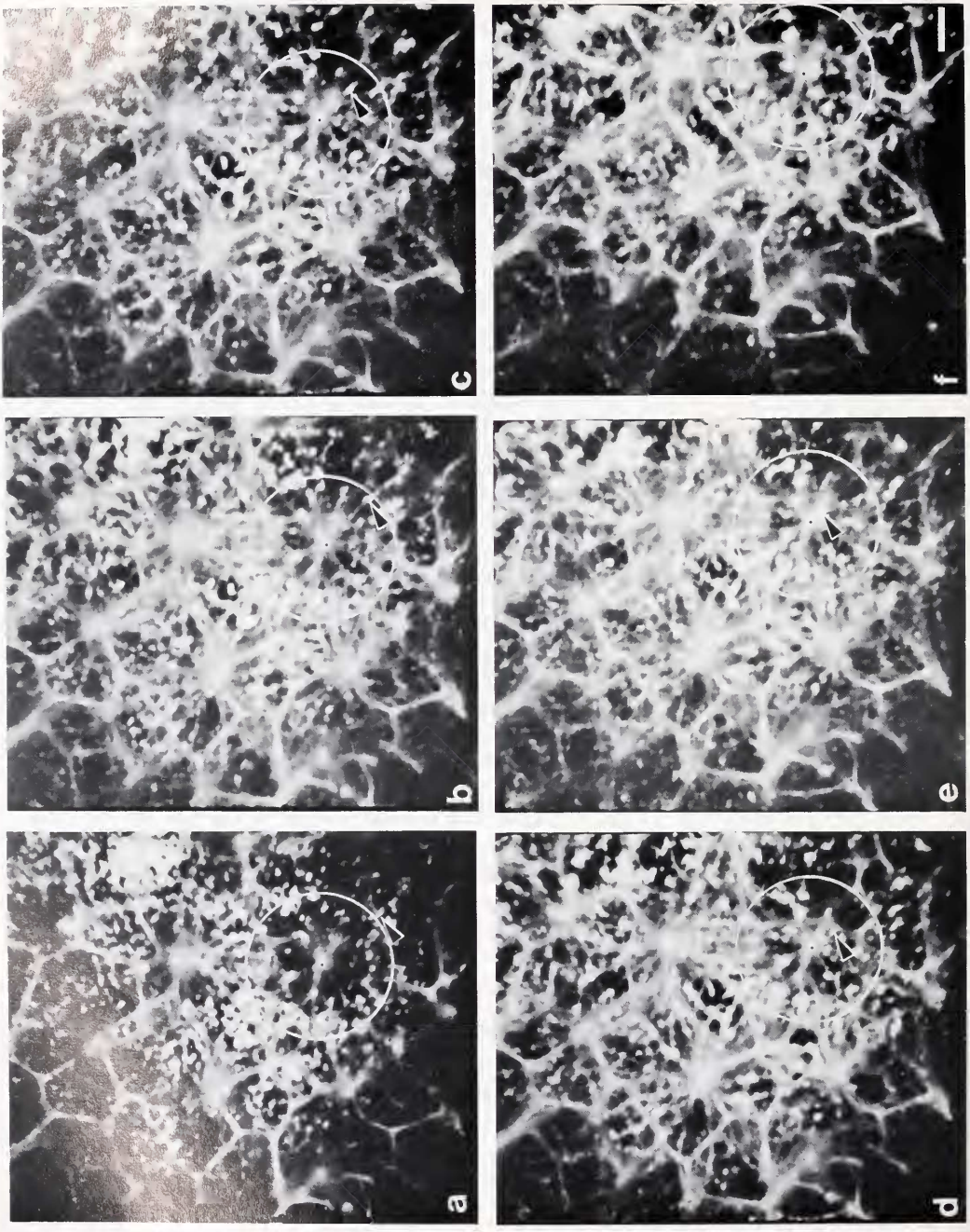


FIGURE 1. Diagram of the sponge surface observed in the film. Note three cell compartments that form the outer dermal membrane of the sponge. Below the dermal membrane is the water-filled subdermal space. The dermal membrane is raised by primary skeletal fibers to form projections on the surface called conules. Secondary skeletal fibers crosslink primary fibers within the sponge interior (skeletal elements indicated by stippled lines). Mesohyl cells form reticulating patterns in the membrane except in areas around oscula (dashed line). Shown are the five conule areas (numbered) and five nonconule areas (rectangles) observed for particle behavior.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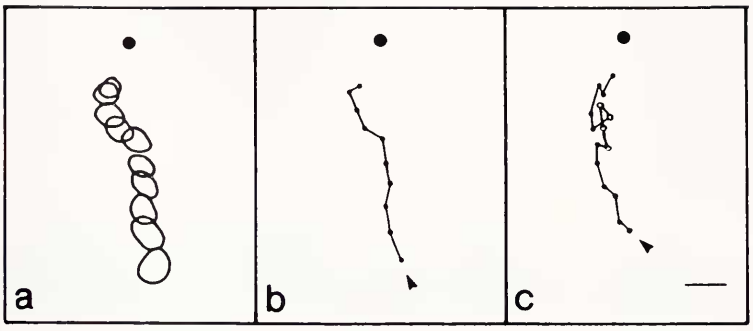

FIGURE 3. Tracings of slow and erratic movements of particles. Shown are two examples of particles moving up radial reticulations to a conule apex (dot), segments equal 10 minutes. (a) Outlines of a moving particle. (b) Linear representation of particle in (a), all movement slow transport. (c) Linear representation of a different particle showing transport interrupted by erratic movement due to dermal membrane contractions (open circles). Scale = 200 μm.

Effects of particles on skeletogenesis

The effects of particle addition and depletion treatments are shown in Figure 6. The addition of particles to explants produced a significant increase in primary fiber length over ambient lengths (Fig. 6a, Table I, $P = .0484$). Although Figure 6a shows a trend of increasing fiber length with increasing particle size, Bonferroni adjusted pairwise *t*-tests showed no significant differences between any two addition treatments (Table I). The effect of depletion of particles on fiber length was also significant (Fig. 6b, Table II, $P = .0004$) and *t*-tests showed that both half caged and fully caged treatments significantly reduced fiber growth (Table II). While cages undoubtedly affected flow rates and light penetration to explants, they did not significantly slow growth of primary fibers (Fig. 6c, Table III, $P = .4682$). Dye releases inside the caged treatments in the field demonstrated the presence of a slow flow which evidently was sufficient to sustain sponges at near normal rates of growth.

In reoriented whole sponges that had been turned on their sides, primary fiber growth gradually became deflected upwards, perpendicular to the former vertical axis of the sponge (Fig. 7). The reoriented fibers were continuous with pre-existing fibers. These whole sponges showed substantial growth of primary fibers up to three times greater than growth in explanted sponges (15 mm in comparison to 5 mm). The increase in primary fiber length created an average percent increase in whole sponge volume estimated at 40% from measurements of Caroplast slabs. Reoriented control sponges subject to ambient particle levels had less upward reorientation of primary fibers, less overall fiber growth, and increased in volume by 26%.

DISCUSSION

Interactions of particles with the sponge surface

The incorporation of particles into sponge skeletons has long been a subject of debate (Schulze, 1879, Lendenfeld, 1889). There was general agreement that sponges

FIGURE 2. Time lapse cinemicrography of particle transport. Movement of a particle on conule 5 shown by the arrow in the circular area; black dot indicates the conule apex. All times are elapsed time after particle application. (a) 25 minutes, dispersed particles clustered at bases of conules. (b) 50 minutes, particle clumping. (c) 90 minutes, slow transport of particle. (d) 97 minutes, rapid displacement of particle by membrane contraction. (e) 150 minutes, continuation of slow transport. (f) 370 minutes, particle has been engulfed in vicinity of apex and is not clearly visible. Scale bar = 1 mm.

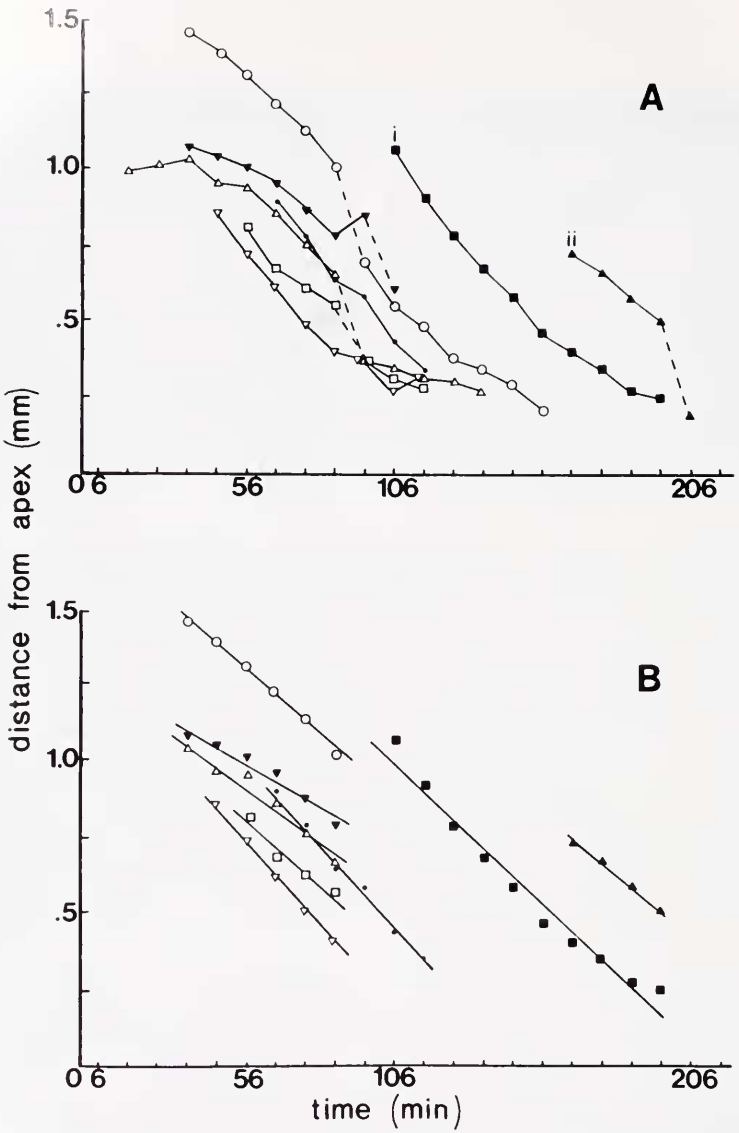

FIGURE 4. Plots of positions of eight particles on conule 5. All particles are moving linearly towards the conule apex (see Fig. 3). (A) Dashed lines indicate erratic movement interrupting slow transport; these were omitted from calculations of transport rate. Note late movement of particles (i) and (ii). (B) Examples of linear regressions to calculate slow transport rate fitted to selected portions of plots in (A).

could select particles since some species appeared to incorporate only foreign spicules in their fibers, but there was disagreement on the mechanism of particle selection. Schulze (1879) suggested that particles were mechanically sorted by physical interactions of the sponge surface with its environment, such as the interaction between surface stickiness and water flow. He suggested that differences in the physical characteristics of sponge surfaces would influence the type of particles incorporated, akin to the deposition of different types of sediments in different parts of a river bed.

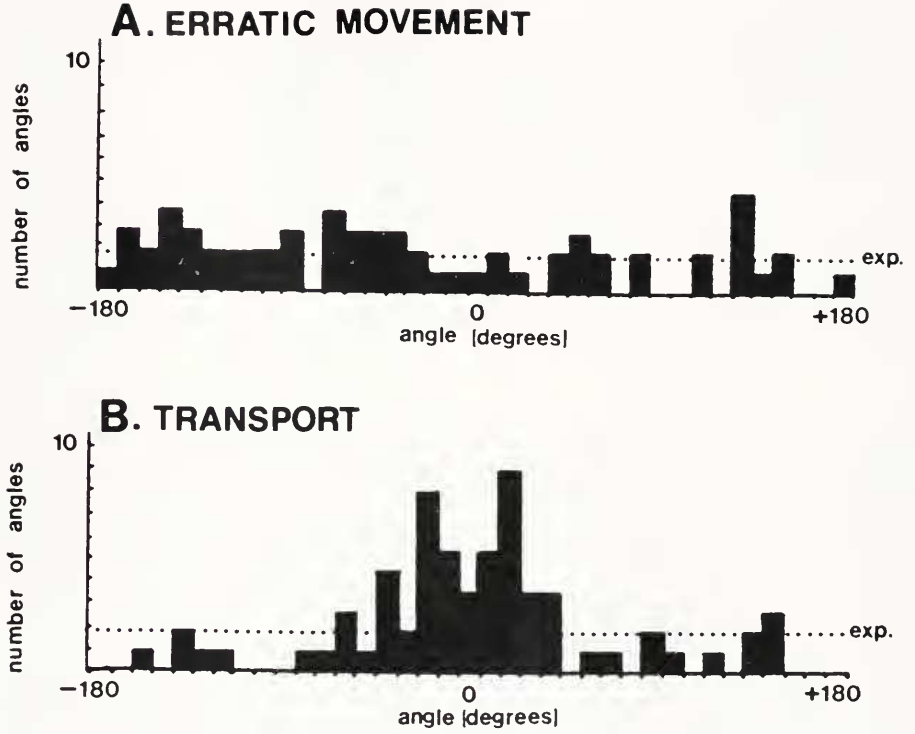

FIGURE 5. Distribution of turning angles for transported and erratic particle movements. (A) Direction of movement for 10 erratic particles (65 angles measured). Expected frequency per angle = 1.76 and indicated by dotted line; $\chi^2 = 34.61$, d.f. = 36, $P < .25$, movement not significantly different from random. (B) Direction of movement for 11 transported particles (72 angles measured). Expected frequency = 1.95, $\chi^2 = 118.14$, d.f. = 36, $P < .001$, movement significantly different from random.

Lendenfeld (1889) argued for a more active selection of particles by the sponge since he observed that a species always incorporated the same kind of foreign material in its fibers, regardless of differences in habitat distribution of individual specimens. Schulze's sorting mechanism insufficiently explained such observations and implied that the nature of particles incorporated into the sponge could change with changing environmental circumstances. However, Lendenfeld's observations lack complete documentation since he did not compare local environmental particle compositions with the composition of particles incorporated in the sponges living in each locality. In addition, these and other studies only speculated on the detailed mechanisms of particle incorporation since they were based on static observations of the particle composition of preserved sponges.

The time lapse observations of the present study support Lendenfeld's views on active selection of particles and provide the first information on the mechanism of particle incorporation by demonstrating the presence of an organized particle transport system on the surface of *D. etheria*. The pattern of transport of particles is correlated with the pattern of reticulations in the dermal membrane but more needs to be known about the factors that determine reticulation patterns and direction of transport on reticulations. Histological studies show that cells of the dermal membrane mesohyl interact with particles and may migrate to transport particles, so regulation of directions of cell migration may determine particle transport patterns (Teragawa, 1985).

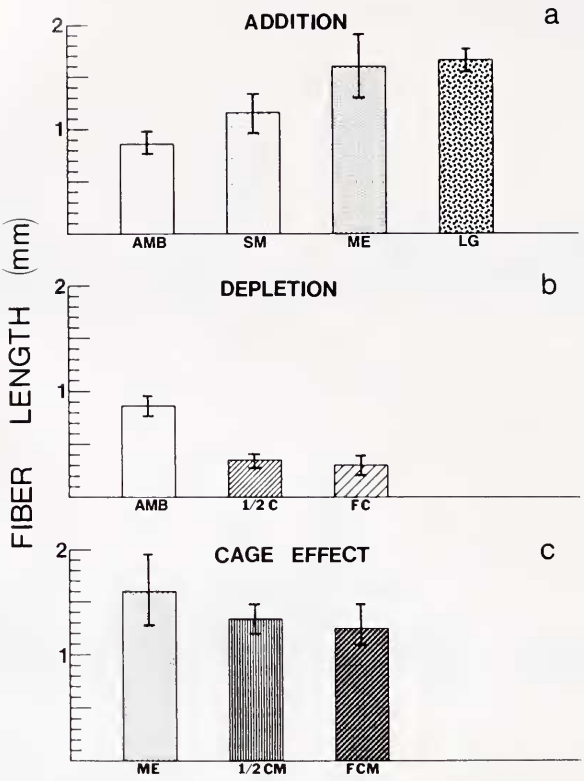

FIGURE 6. Effect of particle availability on primary fiber growth. Mean fiber lengths and associated standard errors of the mean shown for all treatments. (a) Fiber growth with particle additions. AMB = ambient control, SM = small particles, ME = medium particles, LG = large particles. (b) Fiber growth with particle depletions. AMB = ambient control, ½ C = half covered, FC = fully covered. (c) Fiber growth and cage effect. ME = medium particles (uncovered), ½ CM = half covered plus medium particles, FCM = fully covered plus medium particles.

Particle transport is probably only one part of the sponge's response to particle loads. Occasionally, the sponge is likely to receive more particles than it can use, as was the case in experimental manipulations. Not all particles applied were transported and the sponge must be able to clean its surface of excess particles to prevent fouling

TABLE I

One-way analysis of variance of the effect of particle addition treatments on primary fiber length

Source	Sum of sq.	d.f.	Mean square	F	*P*
Particle additions	2.454	3	0.8181	3.08	.0484
Error	5.838	22	0.2654		
Total	8.292	25			

Treatment groups:	AMB	SM	ME	LG[1]

[1] Bar indicates homogeneous subgroups differentiated by Bonferroni multiple comparison tests; abbreviations as in Figure 6.

TABLE II

One-way analysis of variance of the effect of particle exclusion treatments on primary fiber length

Source	Sum of sq.	d.f.	Mean square	F	*P*
Particle exclusion	1.1239	2	0.5619	12.20	.0004
Error	0.8750	19	0.4605		
Total	1.9989	21			
Treatment groups:			AMB 1/2 C FC[1]		

[1] Bar indicates homogeneous subgroups differentiated by Bonferroni multiple comparison tests; abbreviations as in Figure 6.

by other organisms and to allow efficient water exchange. Particle-using sponges like *D. etheria* may thus possess dual and somewhat contradictory responses to particle loads: to retain some particles for use in the skeleton and to eliminate excess particles that cannot be incorporated. Behaviors like the oscillating contractions of the dermal membrane observed in the time lapse filming and the sponge's pumping activities may contribute to elimination of particles.

A summary of the sponge's behavioral responses to a single application of particles can be reconstructed from the time lapse movie results and general observations of particles on sponges (Fig. 8). Percentages were estimated from direct counts of particles in conule and nonconule areas and probably represent maximum values due to the abnormal overloading of the sponge surface with particles. As shown, some particles can be quickly removed from the surface by inhalation through ostia. Other particles retained on the surface for longer time periods are sorted into three groups: particles that are slowly moved off the surface by transport, dermal membrane oscillations, or mucus sloughing; particles that become transported to skeletal fibers; and particles that are directly engulfed without transport. Transport and incorporation of particles into primary fibers are the slowest processes in the sorting of particles on the sponge surface and direct engulfment of particles occurs when these processes are overloaded. Engulfed particles may become incorporated into secondary skeletal fibers forming near the surface since heavily loaded sponges in field growth experiments often had secondary fibers filled with particles (Fig. 7). In normal *D. etheria* skeletons, secondary fibers sometimes contain particles and these may represent particles directly engulfed during periods of heavy particle loads.

TABLE III

One-way analysis of variance of the effect of cage treatments on primary fiber growth

Source	Sum of sq.	d.f.	Mean square	F	*P*
Cage effect	0.4517	2	0.2258	0.78	.4682
Error	6.6228	23	0.2879		
Total	7.0745	25			
Treatment groups:			ME 1/2 CM FCM[1]		

[1] Bar indicates homogeneous subgroups differentiated by Bonferroni multiple comparison tests; abbreviations as in Figure 6.

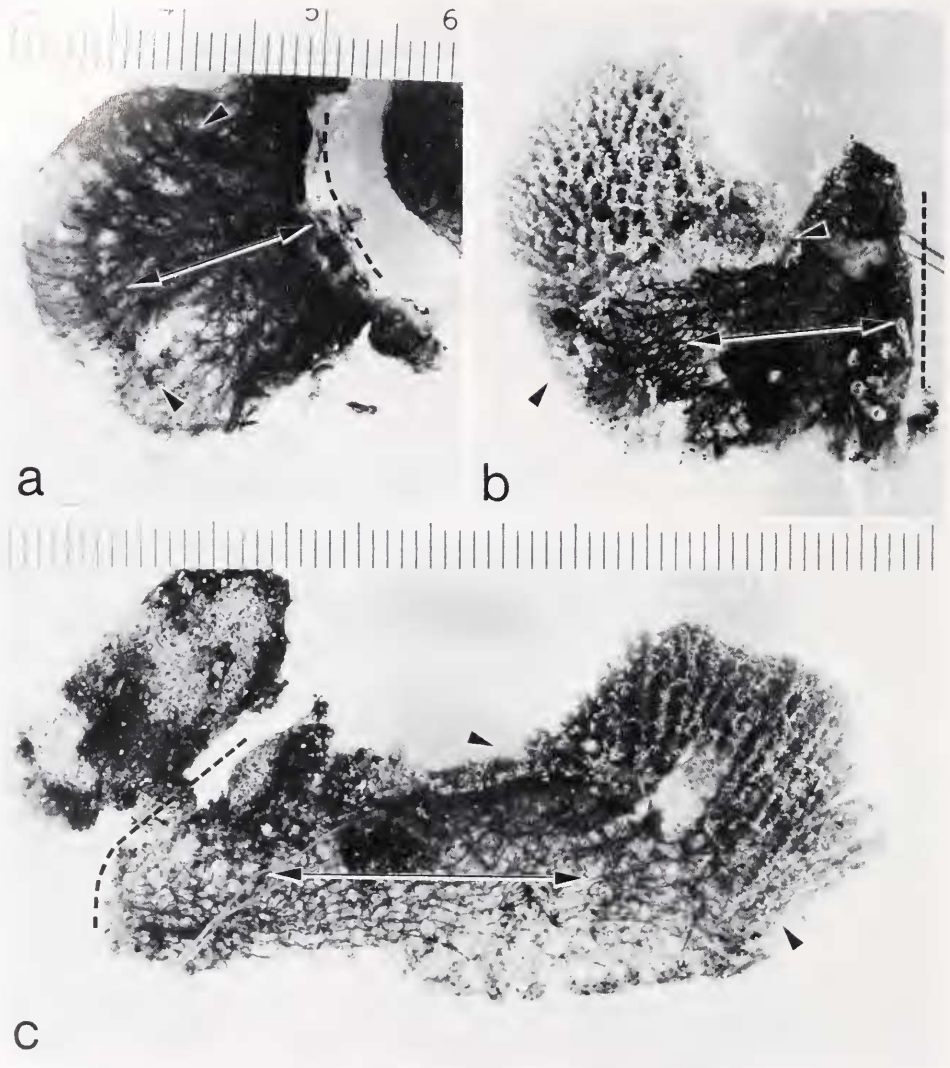

FIGURE 7. Effects of particle treatments on skeletal form. Caroplast sections shown in approximate reoriented positions. Dashed line indicates base of sponge, double arrow indicates original vertical axis. Start of new growth indicated by arrowheads. (a) Cross-section through a reoriented control sponge under ambient particle loads. Arrowheads indicate line of siliceous marker sand. Fiber growth is less and fibers not as strongly reoriented as in treatment sponges. (b and c) Sections through reoriented sponges treated with medium particles. Note how primary fibers smoothly bend upwards; white particles fill the primary and secondary fibers. Scales in mm.

Effects of particles on skeleton growth

The results from the particle addition and depletion experiments on explants show that primary fiber growth can vary with particle availability (Fig. 6). The observations suggest that particles serve as a filler material in the skeleton, allowing the sponge to

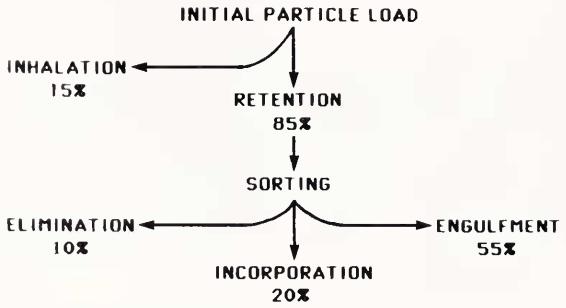

FIGURE 8. Proposed sequence of surface/particle interactions for a single particle application. Percentages are estimated from time lapse film. Smaller particles can be eliminated from the surface by inhalation, remaining particles are sorted on the surface and eliminated, incorporated into skeletal fibers, or directly engulfed into the surface. See text for details.

secrete less material to construct skeletal fibers. Although the correlation between fiber growth and particle size was not found to be statistically significant, the trend of the data suggests that large particles allow a greater increase in fiber length than do small particles, presumably because large particles occupy more fiber volume.

Comparisons of explant and whole sponge growth suggest that the upper limit to fiber growth rate may be controlled by the size of the sponge, since fibers measured from the smaller explants grew less than fibers of the larger, reoriented whole sponges. Larger whole sponges also grew more than smaller whole sponges. A larger sponge may be able to recruit a larger population of spongin-secreting cells to areas of fiber growth due to the sponge's proportionately greater volume, resulting in increased secretion of spongin to consolidate more particles.

Particles were never completely eliminated from the surfaces of sponges in this study, thus the demonstration of an absolute requirement for particles in fiber growth was not possible. However, fiber growth in particle-depleted treatments was significantly decreased to less than half the rate of ambient samples, suggesting that particles have an important role in normal skeletogenesis. No specimen of *D. etheria* was observed to have primary fibers devoid of particles. The incorporation of particles during skeletogenesis may enhance growth rates to allow *D. etheria* to successfully compete for space in the field.

The reorientation of primary fiber growth in reoriented whole sponges may have been caused by a general effect of gravity or light on the sponge or by the specific change in the direction of particle loading. The effects of these parameters could not be clearly separated in the field. However, control sponges exposed to ambient particle loads had fibers that were not as strongly reoriented as fibers of the particle treated sponges (Fig. 7), suggesting that particle load was responsible for the reorientation of primary fiber growth. If gravity or light was the sole influence on fiber orientation, one would expect to see the same amount of fiber deflection in control and treatment sponges, unless reorientation was a function of the amount of growth which differed between the control and treatment sponges. Reorientation of primary fiber growth may be due to skewed patterns of particle transport to primary fibers.

At present it would be premature to generalize about mechanisms of particle incorporation from the example of *D. etheria* alone. Sponges more selective than *D. etheria* in their choice of particles probably have different mechanisms of particle incorporation. More comparative and quantitative studies are necessary to establish the general nature of the transport patterns observed here.

Acknowledgments

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